XPA Gene Mutations Resulting in Subtle Truncation of Protein in Xeroderma Pigmentosum Group A Patients with Mild Skin Symptoms

Yoshito Takahashi[1], Yoko Endo[1], Yoshinori Sugiyama[1], Shintaro Inoue[1], Masahiro Iijima[2], Yasushi Tomita[3], Satoshi Kuru[4], Masahiro Takigawa[5] and Shinichi Moriwaki[5,6]

Comparisons of the clinical manifestations with gene mutations in patients with xeroderma pigmentosum group A (XPA) have suggested that those with mutations closer to the C-terminal coding region of the *XPA* gene have milder neurological and cutaneous symptoms. Here we report on four middle-aged, newly diagnosed Japanese XPA patients whose unusually mild symptoms, especially those affecting the skin, implicate a reduced association of a subtle defect in the C-terminus of XPA protein with skin lesions. All patients had a heterozygous G→C transversion at the splice acceptor site of *XPA* intron 3. We identified previously unreported heterozygous mutations in exon 6: a single-base insertion (690insT) in one patient and a four-base insertion (779insTT and 780insTT) in the other patients. These mutations led to the frameshift that created new premature termination codons, resulting in the production of truncated XPA proteins. They were longer than any previously reported truncated XPA protein, suggesting that the minimal cutaneous symptoms in these patients are due to a higher residual level of XPA protein activity and that the subtle defect in the C-terminus of XPA protein is more closely related to neurological impairment than to cutaneous abnormalities.

Journal of Investigative Dermatology (2010) **130,** 2481–2488; doi:10.1038/jid.2010.137; published online 24 June 2010

INTRODUCTION

Xeroderma pigmentosum (XP) is an autosomal recessive disease characterized by hypersensitivity to sunlight, abnormal pigmentation, and a predisposition to skin cancers, especially on sun-exposed areas (Kraemer *et al.*, 1994; Cleaver and Kraemer, 1995). It results from defective nucleotide excision repair (NER), the system responsible for the repair of UV-induced DNA damage (de Boer and Hoeijmakers, 2000). Cultured cells derived from XP patients have a reduced DNA repair capacity for UV-induced DNA damage and are hypersensitive to being killed by UV (Protic-Sabljic and Kraemer, 1985). Besides the cutaneous manifestation, neurological abnormalities such as loss of hearing, loss of tendon reflexes, walking impairment, and intellectual impairment are observed in about 20% of XP patients (Kraemer *et al.*, 1987). These symptoms are due to progressive degeneration of the central nervous system (Robbins *et al.*, 1991), thought to be caused by a defective repair of lesions that are produced in neurons by reactive oxygen species (Reardon *et al.*, 1997; Rass *et al.*, 2007).

Cell fusion analysis has identified seven complementation groups (A–G) of excision-repair-deficient cells, and there is also a variant form that is proficient in excision repair (Kraemer, 1993; Cleaver and Kraemer, 1995). Patients with xeroderma pigmentosum group A (XPA; OMIM #278700) generally show the most severe symptoms (Takebe *et al.*, 1987) and in most cases die in their second or third decade (Sidwell *et al.*, 2006). The human gene complementing the defect in *XPA* is located on chromosome 9q34.1 and is composed of six exons. The protein consists of 273 amino acids (a.a.; Cleaver *et al.*, 1999) and is required for the early stages of NER, with a role in damage verification and stabilizing of other NER proteins (Berneburg and Lehmann, 2001).

In Japan, the most frequent type of XP is group A (Moriwaki and Kraemer, 2001), and approximately 80% of Japanese XPA patients are homozygous for the G→C transversion mutation of the *XPA* gene at the splice site of intron 3 (IV3: –1 G to C) (Satokata *et al.*, 1990). The other common mutations found in Japanese XPA cases are a nonsense mutation of exon 3, which alters the 116th Tyr codon (Y116stop), and a nonsense mutation of exon 6, which

[1]*Innovative Beauty Science Laboratory, Kanebo Cosmetics, Odawara, Japan;* [2]*Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [3]*Department of Dermatology, Nagoya University Graduate School of Medicine, Nagoya, Japan;* [4]*Department of Neurology, Suzuka National Hospital, Suzuka, Japan;* [5]*Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan and* [6]*Department of Dermatology, Osaka Medical College, Takatsuki, Japan*

Correspondence: Shinichi Moriwaki, Department of Dermatology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki, Osaka 569-8686, Japan. E-mail: der002@poh.osaka-med.ac.jp

Abbreviations: NER, nucleotide excision repair; RFLP, restriction fragment-length polymorphism; TCR, transcription-coupled repair; UDS, unscheduled DNA synthesis; XP, xeroderma pigmentosum; XPA, xeroderma pigmentosum group A

Received 25 December 2009; revised 22 February 2010; accepted 25 March 2010; published online 24 June 2010

alters the 228th Arg codon (R228stop; Nishigori *et al.*, 1993). Including these three mutations, more than 20 mutation sites in the *XPA* gene have been reported worldwide (States *et al.*, 1998; Maeda *et al.*, 2000; Tanioka *et al.*, 2005; Sidwell *et al.*, 2006). The majority are located in exons 3–5, the DNA binding region, at which the mutations tend to occur homozygously in patients with severe manifestations. In contrast, most milder cases have at least one allele with a mutation in exon 6, which interacts with transcription factor IIH (TFIIH) (Park *et al.*, 1995; Nocentini *et al.*, 1997). In extensive comparisons between the distribution of mutations, the various functional regions of the XPA protein, and the severity of clinical symptoms, it was pointed out that the C-terminal domain of the protein has less importance in overall function (States *et al.*, 1998).

In this study, we describe four XP patients with unusually mild cutaneous abnormalities and minimal or late-onset neurological impairment. Surprisingly, they were assigned to complementation group A, despite their surviving to middle age with no skin cancer. In mutational analysis, we found two previously unreported mutations in exon 6 of the *XPA* gene.

RESULTS

Case reports

Case 1 (XP17HM), a 35-year-old man, was referred to the Hamamatsu University School of Medicine, University Hospital, Hamamatsu, Japan. Case 2 (XP21HM), a 30-year-old woman, was referred to the Nagoya University Hospital, Nagoya, Japan. Case 3 (XP42HM), a 40-year-old woman, and case 4 (XP43HM), a 45-year-old man, were referred to the Suzuka National Hospital, Suzuka, Japan. XP42HM and XP43HM are siblings. Each of these patients presented for consultation because he or she had a mild neurological manifestation and a history of moderate sun sensitivity. Their clinical characteristics are summarized in Table 1. Mild dermatological abnormalities, such as mild freckling and telangiectasia on the face, were present in all of the patients (Figure 1). No skin cancer has developed. All of them have microcephaly, intellectual impairment, ataxia dysarthria, involuntary movement, and hyporeflexia (or areflexia). These neurological abnormalities are minimal or were of late onset but are progressing slowly and gradually.

Classification into a mild form of XP on the basis of UV survival and UV-induced unscheduled DNA synthesis assays

UV survival and UV-induced unscheduled DNA synthesis (UDS) of primary fibroblasts from the patients were

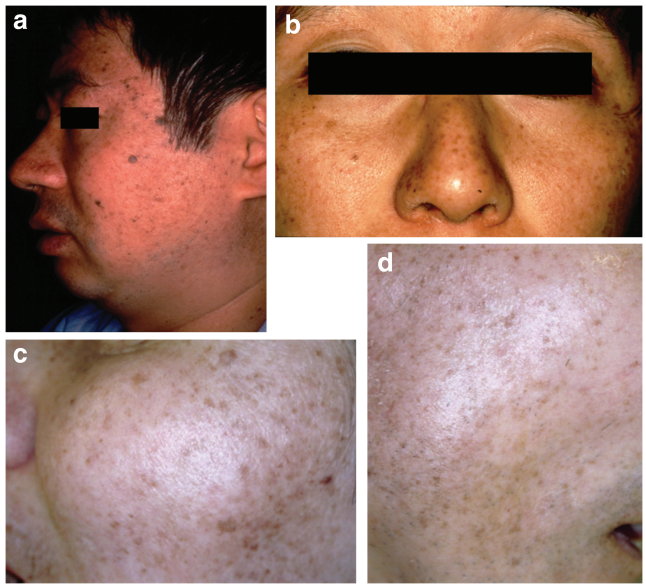

Figure 1. Unusually mild cutaneous features of four patients with xeroderma pigmentosum. (**a**) XP17HM; (**b**) XP21HM; (**c**) XP42HM; (**d**) XP43HM. Each patient's cutaneous symptoms consist only of mild freckling and few, if any, telangiectasias on the face.

Table 1. Clinical and cellular characteristics of patients in this study as well as a typical severe XPA case and normal subjects

Case	Age/Sex	Abnormality in pigmentation	Skin cancer	Neurological abnormality[1]	Fibroblast strain	UDS (%)[2]	UV survival (D_{37}; J m^{-2})[3]
1	35/M	Mild	–	+	XP17HM	17	0.9
2	30/F	Mild	–	+	XP21HM	21	1.4
3[4]	40/F	Mild	–	++	XP42HM	21	0.8
4[4]	45/M	Mild	–	++	XP43HM	32	0.9
XPA[5]	12/F	Severe	–[6]	+++	XP12HM	3	0.4
Normal	42/M	None	–	–	N-42	100	5.9

Abbreviations: F, female; M, male; UDS, unscheduled DNA synthesis; XPA, xeroderma pigmentosum group A.
[1]All the patients have microcephaly, intellectual impairment, ataxia dysarthria, involuntary movement, and hyporeflexia/areflexia. + Mental retardation with minimal, if any, abnormal neurological reflexes; ++ mental retardation and abnormal neurological reflexes; +++ inability to walk because of severe neurological abnormality.
[2]A global ability of nucleotide excision repair system.
[3]UV survival was determined on the basis of colony-forming ability.
[4]Cases 3 and 4 are siblings.
[5]Typical severe XPA patients.
[6]This patient began to protect herself from UV by using sunscreen after she was diagnosed with XPA at the age of 3 years.

compared, according to colony-forming ability (Table 1), with those from a normal subject and a typical XPA patient (XP12HM). The D_{37} (dose that results in 37% cell survival) of cells from the four patients was 0.8–1.4 J m^{-2}, which was much lower than that of cells from a normal subject (5.9 J m^{-2}); however, these cells were less sensitive than those in a typical XPA patient (0.3–0.4 J m^{-2}). The levels of UV-induced UDS in these cells were 14–24% of those of cells from normal subjects, similar to the levels for an intermediate group of XP patients.

Assignment to XP complementation group A

Complementation analysis was carried out with a host-cell reactivation assay in which cells were cotransfected with a UV-damaged luciferase gene expression vector, with expression vectors harboring cloned wild-type XP complementary DNA (cDNA). Increased luciferase activity was observed when the *XPA* gene was transfected into cells from these patients, but luciferase activity was very low in cotransfection with the other *XP* genes (Figure 2a). Thus, only the *XPA* gene complemented the DNA repair defect. This result indicates assignment of these cells to XP complementation group A.

Heterozygous mutations in the *XPA* gene common to Japanese patients

To characterize the mutation of the *XPA* gene in these patients, we first confirmed the presence of the most common Japanese mutation (IV3: –1 G to C) by *Alw*NI PCR-restriction fragment-length polymorphism (RFLP) analysis in amplified DNA fragments of exon 3, including the flanking intron. DNA from these patients showed three bands, whereas two bands appeared in the severe XPA patient, who has this mutation homozygously (Figure 2b). This observation indicates that the patients with mild symptoms had the heterozygous mutation common to Japanese patients. We also examined the other common Japanese mutations (Y116stop and R228stop) using RFLP analysis (Nishigori *et al.*, 1994). However, these mutations were not found (data not shown).

Two insertion mutations in exon 6 of the XPA gene heterozygously

To identify the mutation sites that could not be detected by RFLP analysis, we performed a sequence analysis on each exon of the genomic DNA from these patients. In XP17HM, the heterozygous sequence signal that started from nucleotide 690 in exon 6 was detected (Figure 3a), showing that there was an insertion of a nucleotide (690insT). In the other three patients, the heterozygous sequence signal that started from nucleotide 779 in exon 6 was detected (Figure 3b), showing that there was an insertion of four nucleotides (779insTT 780insTT). These previously unreported mutations are located closer to the C-terminus than any previously reported mutations that cause truncation of the XPA protein

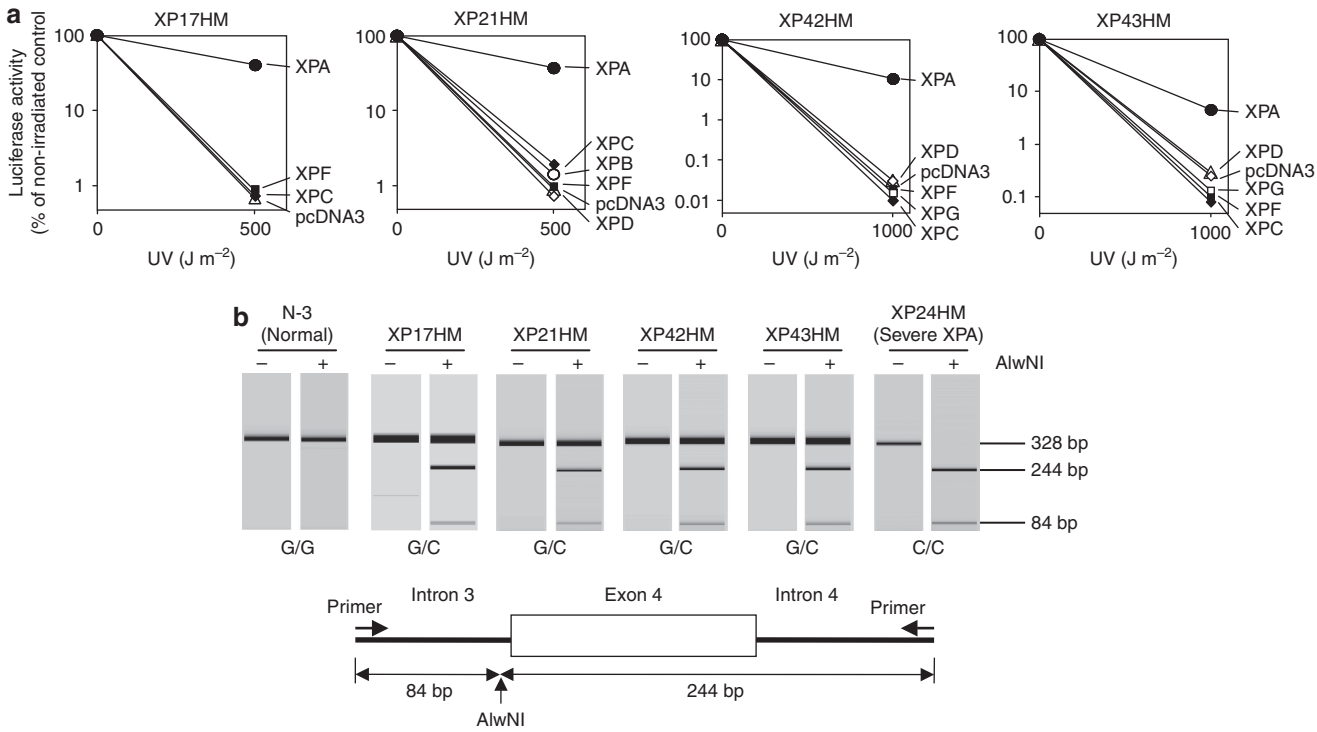

Figure 2. Each patient was diagnosed with xeroderma pigmentosum group A (XPA) and had a common Japanese mutation heterozygously. (**a**) Host-cell reactivation assay was performed via cotransfection of the UV-C-treated reporter plasmid (pGL2Luc) with xeroderma pigmentosum complementary DNA (*XPA* (closed circle), *XPB* (open circle), *XPC* (closed diamond), *XPD* (open diamond), *XPF* (closed square), or *XPG* (open square)) or control plasmid pcDNA3 (open triangle) into fibroblasts from patients. DNA repair capacity was defined as the percentage of residual luciferase activity after repair of UV-irradiated DNA compared with that of nonirradiated DNA. Values taken from two independent experiments are depicted. (**b**) DNA extracted from fibroblasts was analyzed by restriction fragment-length polymorphism as described in Materials and Methods. The polymorphism of patients with a mild phenotype (genotype G/C) showed three bands, whereas normal subjects (genotype G/G) displayed one band and severe XPA patients (genotype C/C) had two.

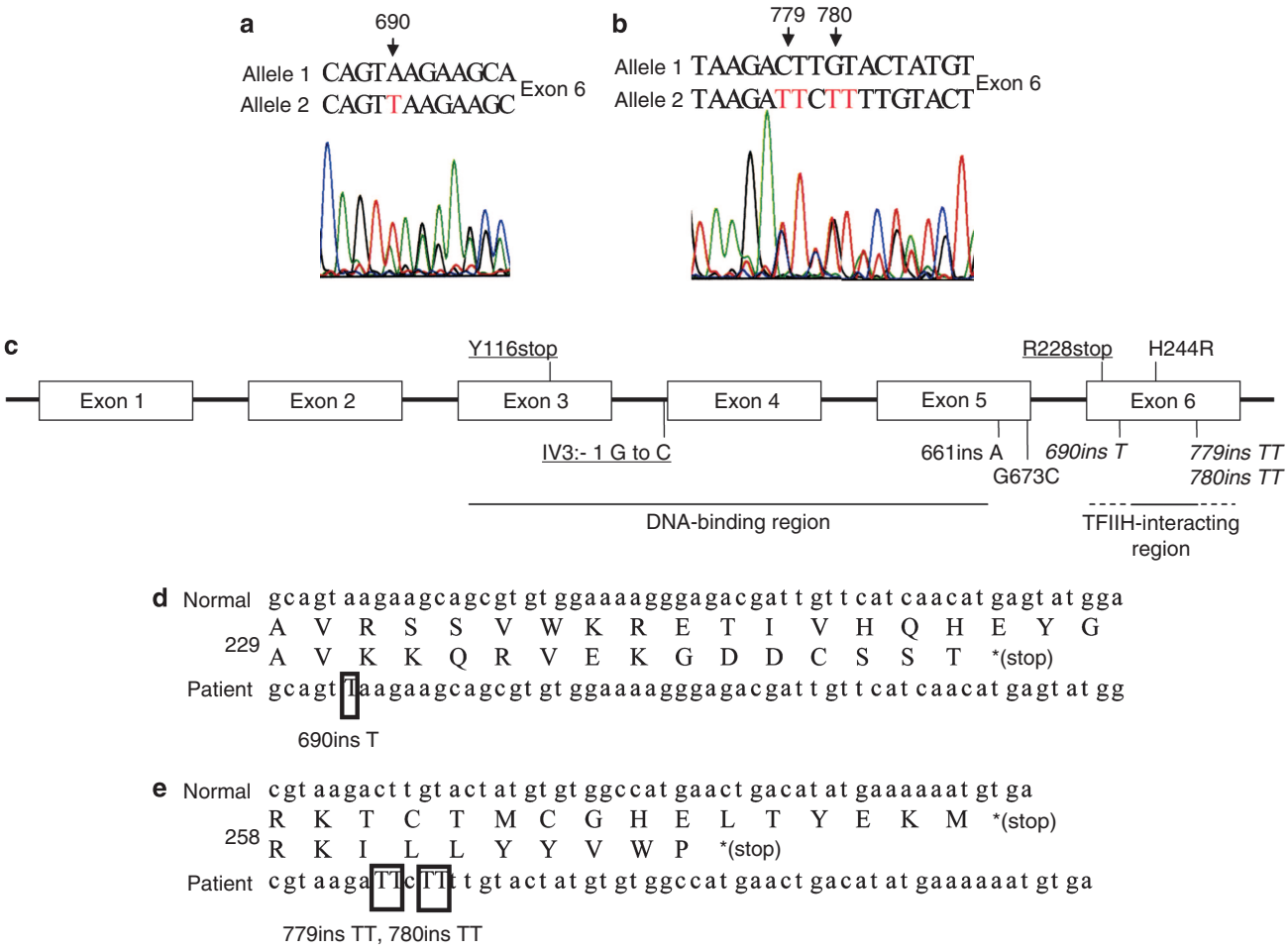

Figure 3. Two insertion mutations were identified in exon 6 of the xeroderma pigmentosum group A (XPA) gene heterozygously. (**a**) The genomic sequencing of exon 6, showing a single-base insertion at nucleotide 690. (**b**) The genomic sequencing of exon 6, showing a four-base insertion at nucleotides 779 and 780. (**c**) Map of *XPA* gene and mutations. The mutations close to the C-terminus reported in the mild cases are indicated. The three Japanese common mutations are underlined. Mutations identified in this study are indicated by italics. Exon 6 is responsible for interacting with transcription factor IIH. (**d**) The base sequences of genomic DNA with coding amino acids corresponding to a normal subject and to a patient with the 690insT mutation. (**e**) The base sequences of genomic DNA with coding amino acids corresponding to a normal subject and to patients with the 779insTT, 780insTT mutation.

(Figure 3c). Mutation 690insT caused a frameshift at amino acid position 231, resulting in a stop codon 15 amino acids downstream (Figure 3d). Another mutation, 779insTT 780insTT, caused a frameshift at amino acid position 260, resulting in a stop codon eight amino acids downstream (Figure 3e). The predicted sizes of XPA proteins are 244a.a. in XP17HM and 267a.a. in the other three patients.

Expression of the truncated XPA protein

We next determined whether the predicted frameshift XPA protein is indeed expressed in cells from these patients. Western analysis revealed two bands as XPA proteins in the cell extract from normal subjects (Figure 4a), consistent with a previous report (Miura *et al.*, 1991). No XPA protein was detected in the severe XPA patient. In each mild XPA patient, a significant band level was detected at a position lower than that for normal protein. The band sizes were estimated at 91% of that of normal protein in XP17HM and 97% of that in the other three patients. These estimated sizes were almost consistent with the predicted size, which was 89% (244/273a.a.) for XP17HM and 98% (267/273a.a.) in the other three patients. To confirm these estimates, we analyzed another patient (XP41HM) who has a nonsense mutation (R228stop) with truncated XPA protein, the predicted size of which is 83% (227/273a.a.) of the normal protein. The estimated size according to western analysis was 82%, showing that the mutations identified in this study cause the truncation of XPA protein. These findings suggest that the unusually mild symptoms might be due to the higher residual level of functional activity of truncated XPA proteins.

Reduced levels of XPA mRNA

To investigate whether the expression levels of XPA proteins correlate with the amounts of XPA mRNA in these patients, we performed northern analysis. Two bands of about 1.3–1.4 kb and 1.0–1.1 kb were detected in the extract from cells of a normal subject (Figure 4b), consistent with a previous study (Tanaka *et al.*, 1990). In the mild XPA patients,

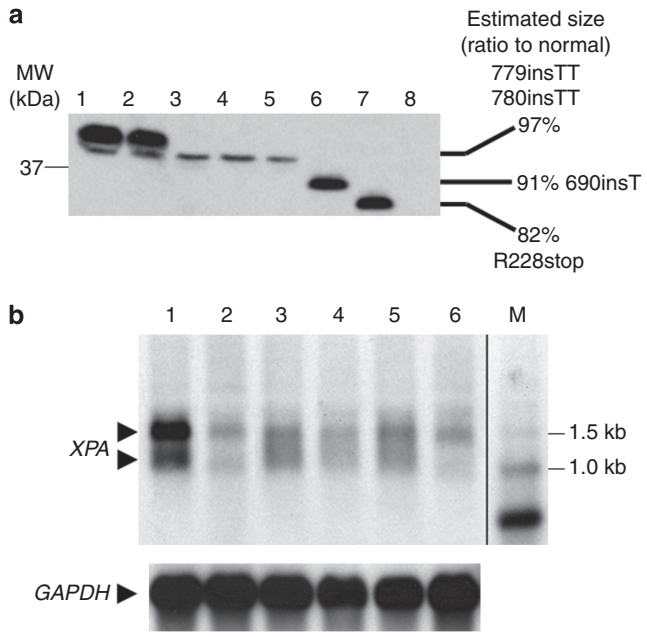

Figure 4. A significant expression of truncated XPA protein and a reduced expression of XPA mRNA in each patient. (**a**) A volume of 50 μg of total protein from N-3 (normal, lane 1), N-75 (normal, lane 2), XP21HM (lane 3), XP42HM (lane 4), XP43HM (lane 5), XP17HM (lane 6), XP 41 HM (mild XPA, lane 7), and XP24HM (severe XPA, lane 8) was analyzed for expression of the XPA protein by western blotting. Equal loading/transfer was confirmed by amido-black staining of membranes. (**b**) A volume of 1 μg of poly $(A)^+$ RNA from N-3 (normal, lane 1), XP17HM (lane 2), XP21HM (lane 3), XP42HM (lane 4), XP43HM (lane 5), XP24HM (severe XPA, lane 6) was subjected to electrophoresis and analyzed by northern blotting with DIG-labeled XPA and GAPDH probe. The GAPDH mRNA is shown as the internal standard. The size of marker RNA is indicated (lane M).

reduced levels of XPA mRNA were detected; these were comparable to those of the smaller mRNA in cells from XP24HM, who has the common Japanese mutation homozygously, suggesting a destabilization of XPA mRNA because of premature termination codons. Therefore, the expression levels of the truncated XPA protein were not relevant to the amounts of XPA mRNA.

DISCUSSION

We have characterized two previously unreported mutations in the *XPA* gene via genetic analysis of four middle-aged XPA patients (XP17HM, XP21HM, XP42HM, and XP43HM). An unexpected finding was that these patients were assigned to XP complementation group A, because, despite being of middle age, they manifested an unusually mild phenotype for XPA, the severest form of XP. Ordinarily, XPA patients are diagnosed on the basis of their severe photosensitivity and developmental delay starting in the first decade of life. The patients in this study have late-onset progressive neurological abnormalities but no cutaneous features other than mild freckles and telangiectasia. Hence, the definitive diagnosis of XP had not been made earlier in their lives.

Thus far, more than 20 mutations of the *XPA* gene have been identified. It is thought that the severity of symptoms of XPA patients depends on the residual activity of the abnormal XPA protein. The typical XPA patients showing both severe neurological abnormalities and extreme photosensitivity have mutations within exons 3–5, almost all of which are deletions or missplicings seriously disrupting the structure. Because these exons encode the DNA binding domain, the severe manifestations are thought to result from the loss of pivotal NER function. Some patients with missplicing in the DNA binding domain show less severe symptoms. It is more likely that these mutations allow the production of a small amount of normal protein by alternative splicing (States *et al.*, 1998).

On the other hand, four mutations close to the C-terminus of the XPA protein have been reported to be associated with milder symptoms (Figure 3c). Two are located in exon 5, but outside the DNA binding region, and the others are in exon 6 (Satokata *et al.*, 1992b; Nishigori *et al.*, 1993; Cleaver *et al.*, 1995; Maeda *et al.*, 1995; Sato *et al.*, 1996). The mutations in exon 5 are an insertion mutation (661insA) and the G→C transversion (G673C) that may allow the production of truncated XPA protein; the protein expression was actually confirmed in a later mutation. The mutations in exon 6 are a nonsense mutation (R228stop) and a missense mutation (H244R). The former is one of the common Japanese mutations. Almost all patients with the above-mentioned mutations close to the C-terminus of the XPA protein were reported to show mild cutaneous symptoms and minimal or late-onset neurological signs. Given that exon 6 interacts with TFIIH, these mutations may cause a decrease in the ability to bind to TFIIH. Therefore, this C-terminal domain is thought to have lesser importance for overall function.

The mutations identified in this study are located closer to the C-terminus than are any previously reported mutations that cause truncation of the XPA protein. They were all compound heterozygote with the most common Japanese mutation (IV3: –1 G to C), which results in severe disruption of the XPA protein, indicating that the newly identified mutations are causative of very mild symptoms. Specifically, the cutaneous symptoms were limited to freckles and telangiectasia and the neurological signs were of late onset and slow to progress. These observations and previous findings suggest that the minimal cutaneous symptoms in these patients are due to the higher residual level of functional activity of XPA protein that has partially lost its ability to interact with TFIIH. In fact, the truncated XPA protein was significantly expressed in each patient, and the truncation of these proteins was much less than that of any XPA protein previously reported (Mimaki *et al.*, 1996; Sato *et al.*, 1996). Thus, the subtle defect in the C-terminus of XPA protein seems to be more closely related to neurological impairment than to cutaneous abnormalities. This possibility merits investigation, e.g., by comparing the ability of truncated XPA proteins to bind to TFIIH in mild cases.

Northern analysis revealed that the amount of XPA mRNA was markedly reduced in all cases in this study, suggesting that XPA mRNA was unstable because of premature termination. However, in XP17HM (690insT), considerable expression of truncated XPA protein was observed, whereas its expression levels were reduced in the

other patients (779insTT, 780insTT). The truncated protein derived from the mutation of 690insT may be difficult to degrade. The reason that XP17HM is not less sensitive than the other patients might be that a shorter length of the truncated protein offsets the greater amount. Whatever the reason, no correlation between the expression levels of the truncated XPA protein and amounts of XPA mRNA was found, implying that each type of truncated XPA has a different level of stability. Therefore, the ability to bind with TFIIH might also be different for each type of mutant XPA protein.

Neurological abnormalities observed in XP patients are postulated to result from the insufficient repair of oxidative DNA lesions in the central nervous system, in which the production rate of reactive oxygen species is higher (Rass *et al.*, 2007). However, a direct correlation between unrepaired oxidative DNA damage and NER deficiency has not been identified. NER consists of two pathways: global genome repair and transcription-coupled repair (TCR). The former repairs nontranscribed DNA lesions throughout the genome; the latter repairs lesions in actively transcribed DNA more rapidly (Moriwaki and Takahashi, 2008). Nondividing neurons in the central nervous system are among the most transcriptionally active cells in the body, suggesting that TCR predominates. Cockayne syndrome, in which TCR is deficient, is known to have neurological defects as a clinical feature but is not characterized by a predisposition to skin cancers (Moriwaki and Kraemer, 2001). These observations imply that TCR is more defective than global genome repair in the studied cases. Therefore, any correlations between the extent of truncation of the C-terminal region and the binding activity of XPA protein to TFIIH may give us insight into the pathogenic mechanisms of neurological defects in XP patients from the view of differences between global genome repair and TCR.

Recently, previously unreported mutations in the *XPA* gene that are associated with the mild phenotype have been identified one after another, including the mutations in this study, thanks to the development of advanced molecular diagnosis techniques. Patients with the mild phenotype show neither skin cancer (this study) nor neurological manifestations (Tanioka *et al.*, 2005; Sidwell *et al.*, 2006) and are unlikely to be diagnosed with XPA in the absence of genetic analysis. The patients in this study are compound heterozygotes for a common Japanese mutation (IV3: –1 G to C) and for previously unreported insertion mutations (690insT or 779insTT, 780insTT). It is known that the homozygote for the nonsense mutation in exon 6 (R228stop) shows a milder phenotype than the heterozygote for this mutation (Satokata *et al.*, 1992a). Therefore, if the homozygote for the mutations we identified exists, it may account for the extremely mild symptoms. It is interesting to analyze the allele frequency of these mutations from the point of view that these mutations may increase the risk for skin and neural aging in subjects who are not clinically recognized as XP patients. If these mutations are found at a comparatively high frequency, they may be used to assess the risk factor of photoaging in a manner similar to the use of single-nucleotide polymorphisms. By contrast, laboratory investigations based on NER abnormality have failed to show clinical and biological differences between XP carriers (heterozygote) and normal subjects (Moriwaki *et al.*, 1993). However, a recent study, in which large-scale screening of Japanese carriers of the founder mutation (IV3: –1 G to C) was performed, revealed that the ratio of Japanese XPA heterozygotes carrying the founder mutation is nearly 1% (Hirai *et al.*, 2006), which is threefold higher than previously estimated (Maeda *et al.*, 1997). In this regard, further analysis of XPA patients with the mild phenotype may provide clues to the relationship between XP mutations and the risk for aging of cutaneous and nervous tissues.

MATERIALS AND METHODS

Cells and media

Cultured fibroblasts designated as XP17HM, XP21HM, XP42HM, and XP43HM derived from cases 1, 2, 3, and 4, respectively, were all established from skin biopsy specimens from the patients. We also used cultured fibroblasts as controls, N-42 and N-75 derived from normal subjects, XP12HM and XP24HM derived from typical XPA patients with the homozygous Japanese common mutation (IV3: –1 G to C), and XP41HM derived from a mild XPA case with a nonsense mutation (R228stop). Analyses were performed with a previously established fibroblast strain (N-3; Moriwaki *et al.*, 1992) and with the nine cell strains mentioned above, after obtaining written informed consent from the donors. The study was approved by the Institutional Review Board of Osaka Medical College and conducted according to the Declaration of Helsinki Principles. The cells were maintained in DMEM (Sigma, St Louis, MO) supplemented with 15% fetal bovine serum (JRH Biosciences, Lenexa, KS) at 37 °C in a 5% CO_2 atmosphere.

Assessment of UV survival

Cells were seeded on 60-mm dishes at 5×10^2–2×10^4 cells per well. After incubation for 18 hours, cells were irradiated by UV and incubated for 1–2 weeks until colonies were formed (with more than 50 cells in a colony). Irradiation was performed with germicidal lamps (GL-10, Toshiba, Tokyo, Japan; predominantly 254 nm) at a dose of up to 20 J m^{-2}, as measured using a UV radiometer (UVR-1, Topcon, Tokyo, Japan). After fixation with formalin and staining with crystal violet, the colonies were counted.

Measurement of UV-induced UDS

Cells were seeded on a glass coverslip in 35-mm dishes. After incubation for 18 hours, cells were UV irradiated with germicidal lamps at a dose of 30 J m^{-2}, followed by incubation with 10 μCi ml^{-1} of methyl-[^{3}H]-thymidine (GE Healthcare, Buckinghamshire, UK) for 3 hours. After labeling, cells were fixed with Carnoy's solution and washed with 5% trichloroacetic acid. The glass coverslips were dipped in nuclear track emulsion, NTB3 (Eastman Kodak, Rochester, NY, USA), for autoradiography. The number of grains per interphase nucleus was scored for 100 nuclei in each specimen. UDS was determined by the percentage of net count when the net count of cells from a normal subject is 100%. Net count is determined by subtracting the mean grain count of nonirradiated cells from that of UV-irradiated cells.

Host-cell reactivation and assignment of XP complementation group

The reporter plasmid, pGL2Luc (Promega, Madison, WI), which harbors the luciferase gene, was used to measure post-UV DNA repair capacity by host-cell reactivation. The reactivation was performed as described previously (Takahashi *et al.*, 2005), with slight modification. Briefly, fibroblasts were seeded on 24-well plates at 2×10^4 cells per well. After incubation for 18 hours, the cells were transfected with the UV-irradiated or nonirradiated plasmid (0.2 μg DNA per well) with the Effectene transfection reagent (Qiagen, Hilden, Germany). After incubation for 48 hours, the luciferase activity in the cell lysate was measured using the PicaGene luciferase assay system (Toyo Ink, Tokyo, Japan). DNA repair capacity was expressed as the percentage of residual luciferase activity after repair of UV-irradiated DNA compared with nonirradiated DNA. In order to assign the patients' fibroblasts to a specific XP complementation group, simultaneous cotransfection was performed with pcDNA3 expression vectors containing different XP cDNAs (*XPA*, *XPB*, *XPC*, *XPD*, *XPF*, or *XPG*) along with the reporter plasmid (Fujimoto *et al.*, 2005).

PCR-RFLP analysis

For detection of the common Japanese mutation (IV3: –1 G to C), PCR-RFLP analysis was performed as described previously (Nishigori *et al.*, 1994). Briefly, DNA was extracted from cells using a QIAamp DNA isolation kit (Qiagen) and amplified by PCR with EX Taq DNA Polymerase (Takara Bio, Shiga, Japan) and primers as follows: sense primer 5′-GGGAATTCTTGCTGGGCTATTTGCAAAC-3′ and antisense primer 5′-GGGGATCCGCCAAACCAATTATGACTAG-3′. The PCR steps consisted of 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 59 °C for 30 seconds, and elongation at 72 °C for 1 minute. Thereafter, the 328 bp PCR product was digested by restriction endonuclease *Alw*NI (New England Biolabs, Beverly, MA) for 3 hours at 37 °C. The G→C substitution creates a new cleavage site for *Alw*NI. The *Alw*NI cuts the 328 bp fragment, resulting in two fragments (84 and 244 bp). The digested PCR products were analyzed by Agilent 2100 Bioanalyzer using a DNA 1000 kit (Agilent Technologies, Santa Clara, CA).

Nucleotide sequence analysis

All six exons and flanking introns of the *XPA* gene in DNA extracted from cells were amplified by PCR with EX Taq DNA Polymerase (Takara Bio), and the PCR products were sequenced using an ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA). The PCR steps consisted of 30 cycles of denaturation at 95 °C for 20 seconds, annealing at 50 °C for 20 seconds, and elongation at 72 °C for 1 minute. Sequencing primers are listed in Supplementary Table S1 online.

Western analysis

The nuclear fraction was extracted from cells using a ReadyPrep Protein Extraction Kit (Bio-Rad Laboratories, Richmond, CA), and the protein concentrations were assayed using a DC Protein Assay kit (Bio-Rad Laboratories). Electrophoresis, transfer, and chemiluminescent detection were performed as described previously (Takahashi *et al.*, 2005). A volume of 50 μg per lane of each extract was run. The transfer membrane (Polyscreen, NEN Life Science Products, Boston, MA) was incubated with 1:1,000 diluted anti-XPA (Clone 12F5, Neomarkers, Fremont, CA) as a primary antibody, followed by incubation with a 1:5,000 dilution of horseradish peroxidase–conjugated goat anti-mouse IgG (H + L; ICN Biomedicals, Aurora, OH).

Northern analysis

RNA was extracted from cells using a Micro-FastTrack 2.0 mRNA Isolation Kit (Invitrogen, Carlsbad, CA). RNA samples were separated by electrophoresis on a 0.8% formaldehyde/agarose gel and transferred to a nylon membrane (Roche, Basel, Switzerland). Preparation of DIG-labeled RNA probe, hybridization, and chemiluminescent detection were performed as described previously (Sayo *et al.*, 2002), with slight modification. The cDNA of human *XPA* genes was generated using reverse transcriptase–PCR with the following primers: sense primer 5′-CATCATTCACAATGGGGTGA-3′ and anti-sense primer 5′-GTCAGTTCATGGCCACACAT-3′. The expected cDNA fragments were ligated into the TA-cloning site of pGEMTeasy (Promega). *In vitro* transcription was performed with cloned cDNA to synthesize antisense RNA probes using a DIG RNA labeling kit (Roche). Membranes were rehybridized with a DIG-labeled glyceraldehyde-3-phosphate dehydrogenase antisense RNA probe prepared from commercially available human glyceraldehyde-3-phosphate dehydrogenase cDNA (Clontech, Palo Alto, CA).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We are grateful to Makoto Tomida, Koichiro Onuma, and Masayo Kaji for technical assistance. We also thank all the patients and their families.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Berneburg M, Lehmann AR (2001) Xeroderma pigmentosum and related disorders: defects in DNA repair and transcription. *Adv Genet* 43: 71–102

Cleaver JE, Charles WC, Thomas GH *et al.* (1995) A deletion and an insertion in the alleles for the xeroderma pigmentosum (XPA) DNA-binding protein in mildly affected patients. *Hum Mol Genet* 4:1685–7

Cleaver JE, Kraemer KH (1995) Xeroderma pigmentosum and Cockayne syndrome. In: *The Metabolic and Molecular Bases of Inherited Disease* (Scriver CR, Beaudet AL, Sly WS, Valle D, eds). McGraw-Hill: New York, 4393–419

Cleaver JE, Thompson LH, Richardson AS *et al.* (1999) A summary of mutations in the UV-sensitive disorders: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. *Hum Mutat* 14:9–22

de Boer J, Hoeijmakers JH (2000) Nucleotide excision repair and human syndromes. *Carcinogenesis* 21:453–60

Fujimoto M, Leech SN, Theron T *et al.* (2005) Two new XPD patients compound heterozygous for the same mutation demonstrate diverse clinical features. *J Invest Dermatol* 125:86–92

Hirai Y, Kodama Y, Moriwaki S *et al.* (2006) Heterozygous individuals bearing a founder mutation in the XPA DNA repair gene comprise nearly 1% of the Japanese population. *Mutat Res* 601:171–8

Kraemer KH (1993) Heritable diseases with increased sensitivity to cellular injury. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KH, eds). McGraw-Hill: New York, 1974

Kraemer KH, Lee MM, Andrews AD *et al.* (1994) The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. *Arch Dermatol* 130:1018–21

Kraemer KH, Lee MM, Scotto J (1987) Xeroderma pigmentosum. Cutaneous, ocular, and neurologic abnormalities in 830 published cases. *Arch Dermatol* 123:241–50

Maeda T, Sato K, Minami H *et al.* (1995) Chronological difference in walking impairment among Japanese group A xeroderma pigmentosum (XP-A) patients with various combinations of mutation sites. *Clin Genet* 48:225–31

Maeda T, Sato K, Minami H *et al.* (1997) PCR-RFLP analysis as an aid to genetic counseling of families of Japanese patients with group A xeroderma pigmentosum. *J Invest Dermatol* 109:306–9

Maeda T, Sato K, Tanaka T *et al.* (2000) Compound heterozygous group A xeroderma pigmentosum patient with a novel mutation and an inherited reciprocal translocation. *Br J Dermatol* 143:174–9

Mimaki T, Nitta M, Saijo M *et al.* (1996) Truncated XPA protein detected in atypical group A xeroderma pigmentosum. *Acta Paediatr* 85:511–3

Miura N, Miyamoto I, Asahina H *et al.* (1991) Identification and characterization of xpac protein, the gene product of the human XPAC (xeroderma pigmentosum group A complementing) gene. *J Biol Chem* 266:19786–9

Moriwaki S, Kraemer KH (2001) Xeroderma pigmentosum—bridging a gap between clinic and laboratory. *Photodermatol Photoimmunol Photomed* 17:47–54

Moriwaki S, Nishigori C, Horiguchi Y *et al.* (1992) Amyloidosis cutis dyschromica. DNA repair reduction in the cellular response to UV light. *Arch Dermatol* 128:966–70

Moriwaki S, Nishigori C, Teramoto T *et al.* (1993) Absence of DNA repair deficiency in the confirmed heterozygotes of xeroderma pigmentosum group A. *J Invest Dermatol* 101:69–72

Moriwaki S, Takahashi Y (2008) Photoaging and DNA repair. *J Dermatol Sci* 50:169–76

Nishigori C, Moriwaki S, Takebe H *et al.* (1994) Gene alterations and clinical characteristics of xeroderma pigmentosum group A patients in Japan. *Arch Dermatol* 130:191–7

Nishigori C, Zghal M, Yagi T *et al.* (1993) High prevalence of the point mutation in exon 6 of the xeroderma pigmentosum group A-complementing (XPAC) gene in xeroderma pigmentosum group A patients in Tunisia. *Am J Hum Genet* 53:1001–6

Nocentini S, Coin F, Saijo M *et al.* (1997) DNA damage recognition by XPA protein promotes efficient recruitment of transcription factor II H. *J Biol Chem* 272:22991–4

Park CH, Mu D, Reardon JT *et al.* (1995) The general transcription-repair factor TFIIH is recruited to the excision repair complex by the XPA protein independent of the TFIIE transcription factor. *J Biol Chem* 270:4896–902

Protic-Sabljic M, Kraemer KH (1985) One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells. *Proc Natl Acad Sci USA* 82:6622–6

Rass U, Ahel I, West SC (2007) Defective DNA repair and neurodegenerative disease. *Cell* 130:991–1004

Reardon JT, Bessho T, Kung HC *et al.* (1997) *In vitro* repair of oxidative DNA damage by human nucleotide excision repair system: possible explanation for neurodegeneration in xeroderma pigmentosum patients. *Proc Natl Acad Sci USA* 94:9463–8

Robbins JH, Brumback RA, Mendiones M *et al.* (1991) Neurological disease in xeroderma pigmentosum. Documentation of a late onset type of the juvenile onset form. *Brain* 114(Part 3):1335–61

Sato M, Nishigori C, Yagi T *et al.* (1996) Aberrant splicing and truncated-protein expression due to a newly identified XPA gene mutation. *Mutat Res* 362:199–208

Satokata I, Tanaka K, Miura N *et al.* (1990) Characterization of a splicing mutation in group A xeroderma pigmentosum. *Proc Natl Acad Sci USA* 87:9908–12

Satokata I, Tanaka K, Miura N *et al.* (1992a) Three nonsense mutations responsible for group A xeroderma pigmentosum. *Mutat Res* 273: 193–202

Satokata I, Tanaka K, Yuba S *et al.* (1992b) Identification of splicing mutations of the last nucleotides of exons, a nonsense mutation, and a missense mutation of the XPAC gene as causes of group A xeroderma pigmentosum. *Mutat Res* 273:203–12

Sayo T, Sugiyama Y, Takahashi Y *et al.* (2002) Hyaluronan synthase 3 regulates hyaluronan synthesis in cultured human keratinocytes. *J Invest Dermatol* 118:43–8

Sidwell RU, Sandison A, Wing J *et al.* (2006) A novel mutation in the XPA gene associated with unusually mild clinical features in a patient who developed a spindle cell melanoma. *Br J Dermatol* 155:81–8

States JC, McDuffie ER, Myrand SP *et al.* (1998) Distribution of mutations in the human xeroderma pigmentosum group A gene and their relationships to the functional regions of the DNA damage recognition protein. *Hum Mutat* 12:103–13

Takahashi Y, Moriwaki S, Sugiyama Y *et al.* (2005) Decreased gene expression responsible for post-ultraviolet DNA repair synthesis in aging: a possible mechanism of age-related reduction in DNA repair capacity. *J Invest Dermatol* 124:435–42

Takebe H, Nishigori C, Satoh Y (1987) Genetics and skin cancer of xeroderma pigmentosum in Japan. *Jpn J Cancer Res* 78:1135–43

Tanaka K, Miura N, Satokata I *et al.* (1990) Analysis of a human DNA excision repair gene involved in group A xeroderma pigmentosum and containing a zinc-finger domain. *Nature* 348:73–6

Tanioka M, Budiyant A, Ueda T *et al.* (2005) A novel XPA gene mutation and its functional analysis in a Japanese patient with xeroderma pigmentosum group A. *J Invest Dermatol* 125:244–6